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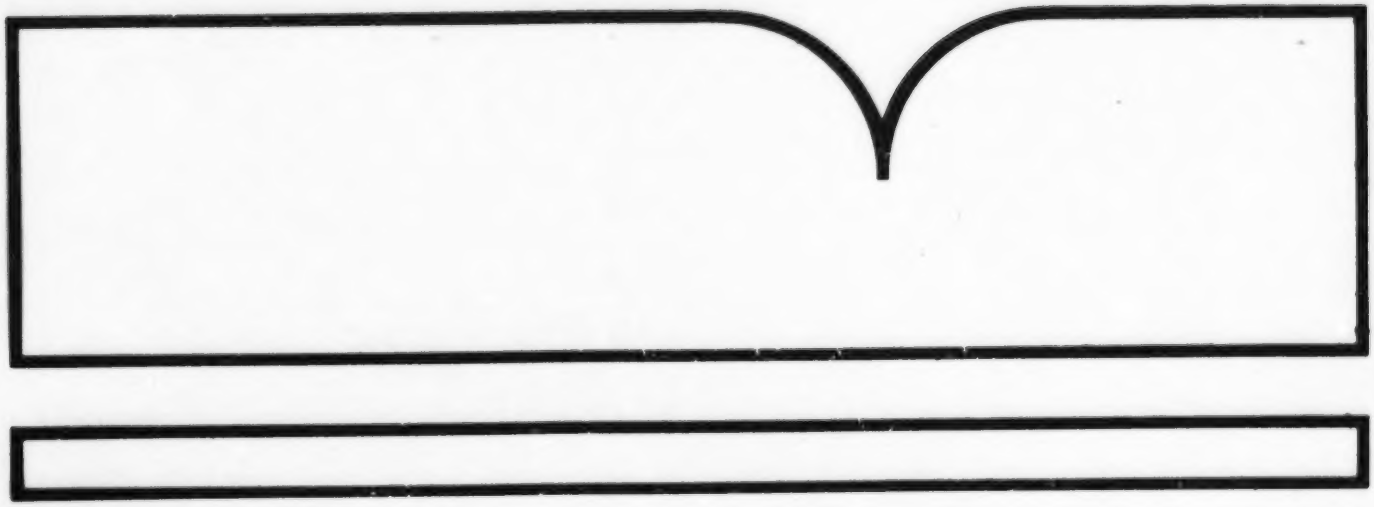

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Determination of Bioactivity of Chemical Fractions of Liquid Wastes Using Freshwater and Saltwater Algae and Crustaceans†

Gerald E. Walsh*

U.S. Environmental Protection Agency, Environmental Research Laboratory, Gulf Breeze, Florida 32561

Richard L. Garnas

U.S. Environmental Protection Agency, National Enforcement Investigations Center, Denver Federal Center, Denver, Colorado 80225

■ Complex wastes from industrial and municipal outfalls were fractionated chemically and tested for toxicity with freshwater and saltwater algae and crustaceans. The organic fraction of each waste was subfractionated into acid-, base-, and neutral-extractable portions, and the inorganic fraction was subfractionated into its anion and cation components. All wastes affected growth of the algae *Skeletonema costatum* (saltwater) and *Monoraphidium capricornutum* (freshwater) or survival of *Mysidopsis bahia* (saltwater) and *Daphnia magna* (freshwater). Usually, bioactivity was limited to one or two subfractions. In some cases, algal growth was stimulated by a fraction or subfraction, whereas stimulation was not detected in whole waste. It is suggested that fractionation must be done in order to estimate the full potential impact of complex wastes on aquatic systems. The method can also be used to identify toxic factors before application of cost-effective control technology.

Introduction

Liquid wastes, composed of mixtures of organic and inorganic substances dissolved and suspended in water, are introduced into freshwater and estuarine ecosystems from municipal and industrial outfalls. The U.S. Department of Commerce and U.S. Environmental Protection Agency (EPA) (*1*) predicted that industrial production will expand dramatically by 2020 A.D. and that the volume of wastes emitted into receiving waters will increase greatly. Since many wastes contain substances that are toxic to aquatic organisms and that stimulate algal growth (*2*), they could cause undesirable changes in the flora and fauna of aquatic ecosystems. Such changes may occur in areas remote from outfalls into rivers or along shorelines of lakes and estuaries, where dissolved and suspended substances may be transported over great distances.

We tested effects of fractions of municipal and industrial wastes on freshwater and saltwater algae and crustaceans. The work was based on the assumption that biological tests on whole waste may not estimate total potential bioactivity because of synergistic, additive, and antagonistic interactions of substances in the mixture. The method may be applied to cost-effective control technology for treatment of complex wastes.

Experimental Section

Chemical fractionation and biological testing were done at the EPA Environmental Research Laboratory, Gulf Breeze, FL, or at Battelle Laboratories at Columbus, OH, and Wareham, MA. Samples of liquid waste were taken from 13 municipal and industrial outfalls in seven eastern and southeastern states. Sampling with an Isco automatic sampler or by grab with a glass bottle was begun in the early morning and continued at 1-h intervals for 23–25 h.

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Approximately 75 L were collected and shipped by air in polyethylene containers to the testing laboratory, where the sample was immediately divided into two portions, one for biological testing of whole waste and one for chemical fractionation. Biological testing and chemical fractionation of whole waste were usually begun immediately after receipt, but when this was not possible, waste was stored in the dark overnight at 4 °C. Fractions were also stored at 4 °C.

The chemical fractionation scheme is given in Figure 1. Biologically active liquid wastes were filtered through a prewashed 1-μm pore size glass fiber filter (Type A-E, Gelman Inst. Co., Ann Arbor, MI) to remove solids and eluted through a column of XAD-4 resin. The efficiency of the resin for removal of organics from aqueous solutions has been demonstrated by others (*3*). The inorganic fraction included all chemicals not adsorbed by the XAD-4 resin but that passed through with the aqueous effluent. This fraction was further treated with a strong-base anion-exchange resin (DOWEX 1-X8) or a strong-acid cation-exchange resin (DOWEX 50W-X8) to generate cation and anion subfractions, respectively, for biological testing. The organic fraction included all chemicals that eluted from the XAD-4 resin with acetone. The organics were further divided into base/neutral, acid, and residual subfractions by using U.S. EPA Method 625 for priority pollutants (*4*). Prior to testing of these fractions, methylene chloride was exchanged with dimethyl sulfoxide (Me_2SO), which is a less toxic solvent in the biological tests. In all, eight fractions may be tested for biological activity by using this scheme.

For each batch of resin used, a volume of deionized water equal to the volume of the test sample was passed through the resin and tested. Toxicity attributable to the fractionation system was never found.

The above scheme was used to test effects of base/neutral, acid, and residual subfractions from a pesticide and plasticizer manufacturing plant. In another series of tests, the base/neutral subfraction was further separated into base and neutral portions by using standard base/neutral partition equilibria. In that case, residuals were not tested for toxicity.

Biological tests were conducted with the freshwater green alga *Monoraphidium capricornutum* Printz (Nygaard) (=*Selenastrum capricornutum* Printz) and crustacean *Daphnia magna* Straus and with the saltwater diatom *Skeletonema costatum* Cleve and crustacean *Mysidopsis bahia* Molenok. Test procedures are given by Duke et al. (*5*). For tests on *M. capricornutum*, whole waste was used to prepare an algal growth medium that contained nutrients (*5*). For *S. costatum*, a test medium was prepared by dissolving commercial artificial sea salt (Rila Products, Teaneck, NJ) in raw waste to 30 parts per thousand salinity and adding nutrients. Dilutions were made with a growth medium prepared with well water (freshwater) or deionized water (saltwater). *D. magna* was exposed to untreated whole waste, and *M. bahia* was exposed to whole waste taken to 30 parts per thousand salinity with artificial sea salt. Dilutions were made with well water or sea salt dissolved in deionized water.

Fractions and subfractions of each waste were used to prepare exposure media. Since the total inorganic fraction was aqueous, an untreated sample was used for exposure of *D. magna*, artificial sea salt was added before exposure of *M. bahia*, and algal growth media were prepared by adding nutrients and salt. The organic fraction and its subfractions were in acetone or Me_2SO, and these were added to exposure systems to desired concentrations.

Table I. Responses of *Mysidopsis bahia* and *Skeletonema costatum* to Subfractions of Liquid Industrial Wastes[a]

plant	*M. bahia* death	*S. costatum* inhibition	*S. costatum* stimulation	active subfractions
gunpowder	–	–	+	anion
titanium oxide	+	+	–	cation
aliphatic amines	–	–	+	anion
oil refinery	+	+	–	neutral organic, cation
tall oil products	+	+	–	neutral organic
phosphoric products	+	–	+	cation, anion
nylon	–	–	+	anion
carpeting	+	+	–	cation
citric acid	–	–	+	anion

[a] + = response; – = no response.

Table II. Responses of *Skeletonema costatum* to Liquid Waste from a Sewage-Treatment Plant[a]

	EC50, %	SC20, %
whole waste	15.4	NE
organic fraction	NE	NE
inorganic fraction	15.5	0.4
cation subfraction	16.5	NE
anion subfraction	NE	0.9

[a] EC50 = calculated concentration that would inhibit growth by 50%; SC20 = calculated concentration that would stimulate growth by 20%; NE = no effect.

Control populations were exposed to the concentration of these solvents used in each test.

Initial range-finding tests, in which concentrations of wastes and their fractions were between 0.1% and 100%, were performed to give a gross estimate of toxicity. Definitive tests were then done with a narrow range of concentrations in order to obtain a more precise estimate. Data reported here are from definitive tests on all species. Algal growth data were analyzed statistically by the method of Walsh et al. (*2*). They are expressed as the 96-h EC50 (calculated concentration of waste that would inhibit growth by 50% after 96 h of exposure) and the 96-h SC20 (calculated concentration of waste that would stimulate growth by 20% after 96 h of exposure). *D. magna* was exposed for 48 h and *M. bahia* for 96 h, and the LC50 (calculated concentration that would kill 50% of the animals) was calculated by probit analysis. Concentration is expressed as percentage of raw waste in test medium or, for fractions, as the percentage equivalent of raw waste.

Results and Discussion

Each waste affected at least one test species. Table I shows results of toxicity tests with marine species on nine outfalls. Wastes toxic to *M. bahia* were also toxic to algae, and some stimulated algal growth at low concentrations. Figure 2 shows results from a paper-products plant and a municipal sewage-treatment plant. Although there was a small loss of toxicity during the fractionation step, bioactive fractions were clearly identified. Also, toxicity of the organic fraction was recovered when the three subfractions were recombined, showing that toxicity was not lost by this process.

Algae were of great value in determining potential effects of the wastes. In addition to responding to toxicants, they responded to growth stimulants (Table I). In several cases, growth stimulation was not found in whole waste but was

Table III. Responses of Freshwater and Estuarine Algae and Animals to Liquid Waste from a Plant That Produced Pesticides and Plasticizers[a]

	estuarine			freshwater		
	S. costatum		*M. bahia*	*M. capricornutum*		*D. magna*
sample	SC20	EC50	LC50	SC20	EC50	LC50
whole waste	<0.1	8.7	0.7	NE	5.6	<1.0
organic fraction	<0.1	7.5	2.9	NE	31.6	<1.0
acid subfraction	0.6	37	13	0.8	60	4.1
base/neutral subfraction	NE	12	8.3	NE	>25, <50	<1.0
residual subfraction	NE	48	NE	NE	NE	NE
inorganic fraction	<0.1	1.7	17	NE	6.2	13
cation subfraction	0.8	51	24	0.8	61	32
anion subfraction	NE	30	28	NE	67	4.5

[a] Concentrations are given as percentage waste; NE = no effect.

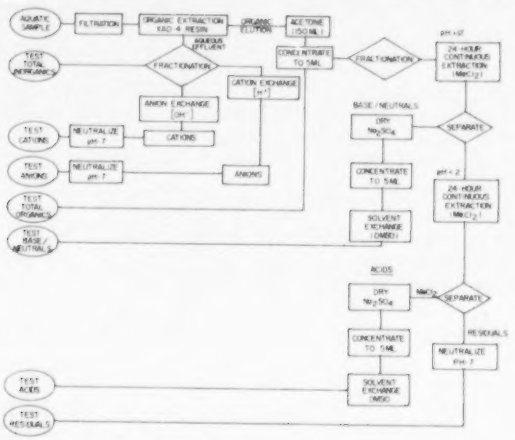

Figure 1. Fractionation and testing scheme for analysis of effects of complex wastes on aquatic organisms.

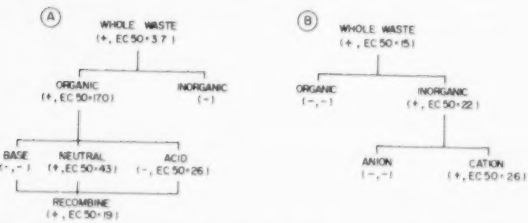

Figure 2. Toxicity of effluents from a paper-products plant (A) and a municipal sewage-treatment plant (B) to *Mysidopsis bahia* and *Skeletonema costatum*: (+) toxic to *M. bahia* (EC50 = calculated concentration that would inhibit population growth of *S. costatum* by 50%); (-) not toxic or stimulatory.

detected after fractionation. Table II shows that whole waste from a sewage-treatment plant inhibited algal growth. The inhibitory factor, probably heavy metals, occurred in the cation subfraction. After fractionation, low SC20 values for the inorganic fraction and anion subfraction were found, demonstrating that interactions between components affect response in toxicity tests on whole waste.

Usefulness of the fractionation scheme in relating gross composition of complex wastes to bioactivity is demonstrated in Table III. This table shows results with waste from a pesticide and plasticizer manufacturing plant, the most toxic waste tested. All fractions were toxic, stimulatory, or both to the freshwater and marine species. Also, whole waste and the organic and inorganic fractions were not stimulatory to *M. capricornutum*, whereas the cation and anion subfractions were highly stimulatory. Generally, marine and freshwater species responded similarly. Some potential effects may be overlooked unless tests on algae and animals are done with fractions, since effects may be masked in tests on whole waste.

Fractionation tests may be used to identify toxic factors before application of waste-treatment systems. For example, if toxicity of a waste were due to heavy metals, treatment technology could be applied to that fraction only, thus reducing the cost of total waste treatment.

Acknowledgments

Figure 1 was drawn by Marcus Cooke of Battelle Laboratories.

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